The Use of Polarography for Automatic Recording in the Chromatography of Proteins

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During the last years several papers have appeared on the subject of protein chromatography [1–4], and more work is in progress. As a part of the work performed in this Institute a method has now been developed for the automatic recording of protein concentration in eluates. Since the adsorption systems mentioned below are not ideal for elution procedures, this paper should be considered mainly as a preliminary report on the method of recording.

The method is an application of the polarographic protein reaction [5,6] to small volumes of liquid emerging from the chromatographic column. All proteins which contain cysteine or cystine give this reaction and can be analysed in this way. * Instead of plotting current-voltage curves, as in the usual polarography, the voltage is fixed at a suitable value, and the current continuously recorded as a function of time. The use of polarography for flowing liquids has been reported by others [7,8], but not for small volumes at variable flow rate, and not together with chromatography.

Except for the adsorption column the apparatus consists of the following parts: a cuvette with a number of drilled holes, a mercury reservoir with rubber tube and capillary, a saturated calomel electrode, an electric circuit including a voltmeter and a microammeter, and a 20 μA strip chart recorder.

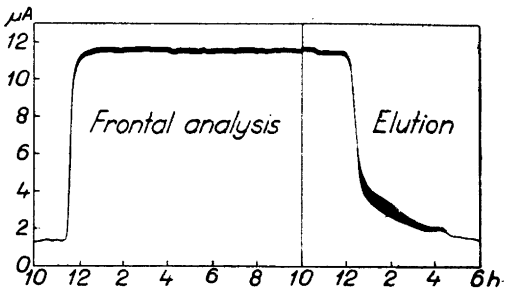

Fig. 1. Frontal analysis of 0.05 % serum albumin, followed by an elution. For data, see the text.

The cuvette is made of perspex (ICI, England) in such a way that all parts are readily visible from outside. Eluates and rinsing solution, if any, are introduced through two openings at one side, while the other side has an opening for the connection to the calomel electrode. The drop tip is introduced from above into the center cylindrical hole, which is vertical and open at the bottom. This hole contains 0.1–0.2 ml of liquid which is held by adhesion. Thus, eluates and mercury leave the cuvette through a common opening. The reason for this arrangement is that it was found impossible to siphon off the mercury through a separate tube because even traces of protein prevent the mercury from forming a coherent body. Instead, a great number of drops will form a plug in the mercury tube, which is then very soon completely shut up.

In order to find out the most suitable regions for the recording, a number of calibrations have been made by manual plotting of current-voltage curves. It was found possible at a dropping mercury potential of −1.7 to −2.0 volts to read protein concentrations as low as about 0.001 %. The most suitable range for recording seems to be 0.005–0.1 % which means that the method may be considered a micro one. Above 0.1 %, however, the

* Of course, if proper modifications are introduced, the method described here may be used also for other substances than proteins.

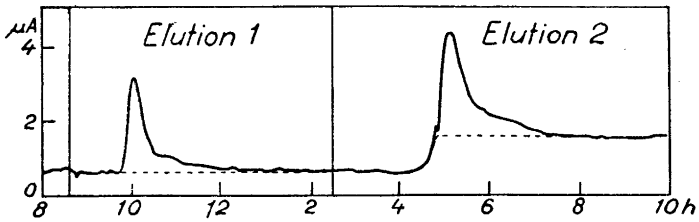

Fig. 2. Stepwise elution of 0.2 mg each of serum globulin and serum albumin. The base line, above which the protein concentration is recorded, has been drawn as a dashed line. For data, see the text.

current-concentration curves are very curved and the method is not well suited for accurate recording.

As the method is intended also for use in salting out adsorption [1, 2], some calibrations were made at high salt concentrations. Even in this case the sensivity is high; for example it is possible to detect a protein concentration of 0.003 % in 80 % saturated phosphate buffer. This means that the specificity is greater than in the interferometric method, where a minute variation of the salt concentration during a single run will completely mask the variation in protein concentration.

As examples of what the method can perform, some curves reproduced from actual strip chart records are given. Fig. 1 shows a frontal analysis of 0.05 % serum albumin in 0.35 *M* phosphate buffer (pH 6.7) on Hyflo Super Cel in a 1000 π cmm. (3.1 ml) perspex filter. The frontal analysis (total, 21 ml) is followed immediately by an elution (total, 8 ml) with the solvent. In this case the adsorption is very small, and probably this system could be used to separate proteins from other substances which are adsorbed at the conditions given. Fig. 2 shows the stepwise elution of serum albumin and serum globulin from a 2000 π cmm. (6.3 ml) column of a mixture (1+4) of tricalcium phosphate * and Super Cel. The globulin was eluted with 21 ml of 0.035 *M* phosphate buffer and the albumin with 20 ml of 0.105 *M* phosphate buffer, both at pH 6.7. The peak concentrations in the two elution zones were estimated to about 0.02 %.

This work is being continued in order that the method can be made more generally adaptable as a technique for analysis and separation of proteins. A report in greater detail will be published later.

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